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(57) Abstract

The present invention comprises a method for altering the structure or composition of wood which method comprises adding to compressed wood a medium comprising a structure-altering or composition-altering effective amount of at least one fungi or bacteria, one or more culture products thereof, such as enzymes, one or more substances obtained therefrom, one or more enzymes from non-microbial sources, or combinations thereof, to produce inoculated wood upon decompression. Compression of the wood substrate prior to or during application of the biological agent introduces fractures in the wood substrate thereby allowing the biological agent to penetrate the wood structure. Furthermore, the biological agent is drawn into the wood substrate as the wood re-expands during decompression.

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IMPROVED METHOD FOR BIOLOGICAL PRETREATMENT OF WOOD CHIPS

The present invention relates to a method for the treatment of wood pulp and other wood materials, and more particularly using fungi, culture products thereof such as enzymes, or substances therefrom to inoculate compressed substrate.

The present invention has particular application in the preparation of wood materials, such as wood pulp. Wood is comprised in large part of cellulose and hemicellulose fiber and amorphous, non-fibrous lignin. The lignin functions to hold the fibrous components together. During the treatment of wood to produce wood pulp, the wood substrate is converted to a fibrous mass by separating the fiber either chemically or mechanically. Such processes are involved in the preparation of paper and paper products in which, generally, the wood chip starting material is transformed into a fibrous mass of wood pulp during the pulping process. The pulping process may be carried out by a variety of mechanical, chemical or biological methods.

It is known in the art to use biological products, e.g., microorganisms including fungi and bacteria, the culture products of such microorganisms, including enzymes, and substances extracted from such culture products, to degrade wood chips and other wood materials. Myers, G.C. et al., "Fungal Pretreatment of Aspen Chips Improves Strength of Refiner Mechanical Pulp", Tappi Journal, 105 (May 1988). In the case of wood chips, the structure may be selectively decomposed by the appropriate application of genera or species of fungi having greater or lesser activity for degrading lignin, cellulose and hemicellulose. Nishida et al., U.S. Patent 5,081,027, for example, disclose a method of producing wood pulp using cultured microorganisms with high ligninolytic enzyme production and activity to decompose high levels of lignin in the wood chip substrate.

Nishida et al., supra, disclose that various strains of the genera *Phanerochaete* and *Coriolus* have high ligninolytic activity. Particular strains of *Phanerochaete chrysosporium* with desirable ligninolytic activity are disclosed, for example, by Buswell et al., U.S. Patent 4,889,807. Paice et al., U.S. Patent 4,830,708, teach the use of fungi, such as species of *Phanerochaete*, *Coriolus*, and *Pleurotus*, for the degradation of wood substrates.

Commercial applications of fungal degradation of wood chips has traditionally involved application of a solution or suspension of fungi or fungal spores by spraying or

soaking. This results in non-uniform distribution of the fungal culture. After such surface inoculation of the wood chip substrate, significant time is required to effect the alteration of the structure and composition of the wood. The time is a function of the need for the fungi to grow or fungal spores to germinate and grow throughout the wood substrate medium.

Conventionally, the incubation time is at least two weeks, and more frequently, is substantially longer, for example two to four weeks. During this time, valuable wood substrate is tied up and not available for further conversion into pulp and valuable process space is occupied by slowly denaturing piles of wood chips.

The present invention alleviates these problems by accelerating the incubation process, or by eliminating the need for growth of the fungi by application of enzymes, thereby speeding up the production of denatured wood; i.e., conversion of wood chips to wood pulp. This is accomplished through recognition that the rate limiting step in the process of fungal or enzymatic degradation of wood substrate is the time required for the fungal culture to grow through or for the enzyme to penetrate the wood chips to reach sufficient portions of the interior of the wood chips. By means of the process of the invention, the biological agents, such as fungal or bacterial culture, culture products thereof, such as enzymes, substances produced therefrom, and/or enzymes from non-microbial sources, are delivered into the substrate and are not merely applied to the surface. In the process of the invention, nutrients, growth stimulators, buffers, surfactants and other promoters are likewise deliverable into the substrate with the biological agent as desired.

One advantage of the present invention is that it provides an accelerated, more efficient means of converting wood chips to wood pulp. A further advantage is that the process allows for recovery and recycling of the biological product solution or suspension. Another advantage of the invention is the removal of undesirable resins and other extractives which are expressed during the compression step, separated from the wood chip and thereby removed from subsequent steps of the pulp refining and paper manufacturing process.

SUMMARY OF THE INVENTION

The present invention comprises a method for altering the structure or composition of wood which method comprises adding to compressed wood a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent which may comprise one or more fungi or bacteria, one or more culture products thereof, such as enzymes, one or more substances obtained therefrom, one or more enzymes from non-

microbial sources, or combinations thereof, to produce inoculated or impregnated wood upon decompression. Compression of the wood substrate prior to application of the biological agent introduces fractures in the wood substrate thereby allowing the biological agent to penetrate the wood structure. The biological agent is drawn into the wood substrate as the wood re-expands during decompression. Furthermore, the biological agent may be drawn into fissures in the wood substrate by capillary action after decompression.

In one embodiment of the invention, the wood comprises wood chips and the method is useful in the production of wood pulp. The wood may be compressed by any suitable means, such as, for example, by means of a compression screw device or a roll press device. The wood is subjected to pressure in the range from about 200 to about 10,000 psig; the pressure may be applied for about 0.1 to about 60 seconds. In a further embodiment, the wood may be subject to a pretreatment step to remove undesirable microorganisms and other contaminants prior to inoculation with the biological agent. This pretreatment step may occur prior to compressing the wood. Preferably, the pretreatment step comprises subjecting the wood to steam immediately prior to the compression step. In addition to steam, other pretreatment agents may include hot water, dry heat, gamma, ultraviolet radiation, infrared radiation and gas treatment.

The biological agent preferably comprises one or more fungi or bacteria, one or more culture products thereof, such as enzymes, one or more substances obtained therefrom, one or more enzymes from non-microbial sources, one or more chemically modified enzymes, or combinations thereof. The fungi or bacteria utilized in the process of the invention may comprise lipid-degrading, protein-degrading, lignin-degrading, cellulose-degrading, or hemicellulose-degrading fungi, bacteria or combinations thereof, either as combinations of species or strains of fungi or bacteria or as individual fungal or bacterial species or strains with multiple functionalities. Suitable fungi may be selected from the group consisting of the genera *Ceriporiophsis*, *Phanerochaete* and *Ophiostoma*. The biological agent may alternatively or additionally comprise culture products produced by fungi or bacteria cultures, or the substances obtained from such culture products, for example enzymes, such as lipolytic, proteolytic, ligninolytic, cellulolytic and hemicellulolytic enzymes, e.g., lipase, protease, cellulase, hemicellulase, ligninase and laccase. Alternatively, enzymes from non-microbial sources, such as porcine pancreatic lipase, could be use in place of or in conjunction with microbial cultures or culture products.

The inoculating or impregnating medium of biological agent is preferably maintained at a temperature in the range of about 10° to about 100°C, and a pH in the range from about 2 to about 11. The medium may further comprise additional compounds suitable as nutrients or growth stimulators for the fungi or bacteria, as buffers, salts, or co-factors for the enzymes, or as dispersion agents to promote distribution of the fungi or other biological agent throughout the wood. Preferably, the wood chips or other wood substrate material is contacted with excess medium for about 5 to about 60 seconds. The excess medium is then removed from the wood chips and either discarded or reused in further inoculation of wood chips. Alternatively, especially when enzymes are used, the wood chips or other wood substrate material is contacted with excess medium containing the biological agent for extended periods of time from 60 seconds to two weeks. The excess medium is then removed from the wood chips and either discarded or reused in further inoculation of wood chips.

The inoculated wood chips are incubated for a sufficient period of time to alter the structure or composition of the wood to the desired state; preferably the incubation period lasts for about 60 seconds to about 14 days. The duration may be adjusted to accomplish a desired degree of decomposition of lipid, protein, lignin, cellulose or hemicellulose, or combinations thereof. Alternatively, when enzymes are used, there may be no measurable removal or decomposition of lignin, cellulose or hemicellulose, or combinations thereof; rather, only a small portion of the covalent bonds of these wood constituents may be hydrolyzed.

DETAILED DESCRIPTION

The present invention comprises a method for altering the structure or composition of wood material which method comprises adding to compressed substrate, e.g., wood chips, a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent comprising one or more fungi or bacteria, one or more culture products thereof, such as enzymes, one or more substances obtained therefrom, one or more enzymes from non-microbial sources, or combinations thereof, to produce inoculated or impregnated substrate upon decompression. Compression of the substrate prior to application of the biological agent introduces fractures and fissures in the substrate thereby allowing the biological agent to penetrate the structure of the substrate. Biological agent is drawn into the substrate as the substrate re-expands during decompression as well as by capillary action. If the biological agent is contacted with partially or fully decompressed substrate, the biological

agent will, to a lesser or greater extent, be drawn into the substrate through the fractures and fissures by means of capillary action.

The process of the present invention will be described primarily in the context of converting wood chips to wood pulp for use, for example, in the paper manufacturing industry. However, it is recognized and understood that the process of the present invention is applicable as well to a wide variety of applications in which biological agents, including, but not limited to, fungi and bacteria, or their culture products or substances obtained therefrom, such as enzymes, or enzymes from non-microbial sources, are the active agents in the desirable degradation of wood materials. Likewise, the process of the present invention will be described primarily with regard to use of biological agent comprising fungi or bacteria, culture products thereof, and substances obtained therefrom, such as enzymes, or enzymes from non-microbial sources, or combinations thereof.

The process of the present invention provides a method for altering the composition and structure of wood chips and other wood material substrates using a medium comprising one or more biological agent which agent comprises one or more naturally-occurring or bioengineered strain of fungi or bacterial, culture products of such fungi or bacteria, or active substances produced in and extracted from such biological cultures, such as enzymes, or enzymes from non-microbial sources, or combinations thereof. The medium comprising biological agent is applied to the wood material substrate, for example wood chips, after the substrate has been compressed to a sufficient degree to introduce fractures and microfissures or the like in the substrate material.

According to one embodiment of the invention, the substrate is decompressed during or after inoculation or impregnation of the substrate with biological agent. In another embodiment of the invention, the inoculation or impregnation step occurs after decompression of the substrate and the medium comprising biological agent is drawn in by capillary action to the fractures and microfissures introduced during the compression step. Capillary action will occur in the inoculation/impregnation process in the former embodiment as well. After inoculation or impregnation of the substrate by such medium, the inoculated or impregnated substrate is incubated for a sufficient period of time for the biological agent to effect the desired degree of degradation of the substrate's composition and/or structure. The terms "inoculation" and "impregnation" are used to refer to contacting substrate with living biological agents and non-living compositions respectively; e.g., fungi cultures and enzyme

solutions, respectively. The terms are otherwise interchangeable when describing the process of the invention.

Compression of the wood substrate to form compressed wood may be accomplished by any suitable means known or developed in the art. Compression of the wood is intended to provide a substrate which, after addition of the biological agent and when allowed to decompress and/or by capillary action, will draw the medium comprising biological agent into the interior of the wood, thereby enhancing distribution of the active biological agent throughout the wood substrate. Preferably, the degree of compression is sufficient to disrupt the structure of the wood to introduce fractures or microfissures in the wood. Such fractures or microfissures serve to further enable distribution of the biological agent uniformly throughout the wood. The wood is subject to pressure in the range of 200 to 10,000 psig; preferably 400 to 8,000 psig. Typically, the pressure is applied for a sufficient time to compress the wood and create the desired fissures and fractures in the wood as described previously; typically, at least about 0.1 seconds, preferably 0.1 to 60 seconds.

At a minimum, the pressure employed is sufficient to induce cracks and fissures in the wood chips and may collapse airspaces in the wood chip and the individual fibers. This allows for direct access of the impregnation solution into the chips and fibers after release of the pressure and during the resulting mechanical expansion of the chips and fibers. The impregnation solution is drawn into the voids created in the inner chip and fiber structure upon release of the pressure. The fissures may also serve to draw in the impregnation solution by capillary action. The maximum pressure employed is adequate to produce the results described above, but not such as to collapse the entire structure of the wood chips and damage the fibers. Determination of suitable compression pressures for various wood chip substrates is within the ability of the skilled practitioner in the art.

In the context of the paper manufacturing industry, the wood chip substrate may be subjected to pressure, and hence compressed, by means of a roll press device or a screw compression device. An example of the latter is the IMPRESSIFINER® compression device which is currently used in the industry to press wood chips to affect pulp quality and which may reach pressures in excess of 6000 psig using a 4:1 compression ratio. The IMPRESSIFINER apparatus is particularly suitable for use in the process of the present invention because it is readily adaptable for application of the biological agent to the compressed wood chips as they are discharged from the barrel of the device. By analogy, the medium comprising biological

agent may be added to the compressed wood chips in lieu of the water or chemical solutions which may typically be applied at that point in the pulping process. See, Leask, R.A., "Groundwood - Chip", pp.190-119, in Britt, K.W., Ed., Handbook of Pulp and Paper Technology, 2d ed., Van Nostrand Reinhold Company, New York (1970). However, the process of the present invention is not limited to any particular compression device; the practitioner in the art may employ any suitable means for subjecting the wood substrate to pressure.

In the process of the present invention, during or after compression of the wood, for example as the wood is discharged from the compression device, it is contacted with medium comprising biological agent. In one embodiment of the invention, the compressed substrate is relaxed to produce fully or partially decompressed wood prior to contacting with medium comprising biological agent. In this embodiment, the medium is drawn into the substrate by means of capillary action into the fractures and microfissures in the substrate.

As noted above, the medium comprising biological agent may comprise a solution or suspension of one or more strain of fungi or bacteria, culture products of such fungi or bacteria, such as enzymes, active substances produced in and extracted from such fungal or bacterial cultures, enzymes from non-microbial sources, or combinations thereof. The medium is preferably maintained at a temperature in the range of 10° to 100°C and at a pH in the range of 2 to 11. Selection of suitable temperature and pH within those ranges depends in turn on the selection of biological agent used in the medium; such determination is within the ability of the skilled practitioner.

The compression step in the process of the invention provides another advantage of the present invention over the prior art. During the compression step, resins and other extractives may be expressed from the wood substrate. These compounds are undesirable in the pulping process and are separated from the wood chips prior to the inoculation step according to the process of the invention. They are, thereby, removed from subsequent steps of the pulp refining and paper manufacturing process.

The wood is preferably contacted with the medium for sufficient time and in the presence of sufficient quantity of biological agent, such that a structure-altering and/or composition-altering effective amount of biological agent is added to the wood; i.e. is absorbed into the wood substrate at least in part by being drawn into the fractures and microfissures in wood as the wood decompresses or by capillary action. Preferably, the compressed wood is

contacted with the medium of biological agent for at least about 5 seconds, more preferably about 5 to about 60 seconds. This forms the inoculated wood.

In order to ensure that sufficient quantity of biological agent is added to the wood to comprise a structure-altering and/or composition-altering effective amount, the biological agent is preferably added in excess. This may be accomplished, for example, by adding the compressed wood or the decompressed wood to a solution of biological agent or by pouring or spraying copious amounts of biological agent on to the compressed wood. For example, the compressed wood chips may be delivered from the compression device into a vat or similar container which contains, or to which is subsequently added, the medium comprising active biological agent. Following addition of the biological agent to the compressed wood, the inoculated wood is incubated to convert the wood to wood pulp.

Regardless of the means employed, an excess of a structure-altering and/or composition-altering effective amount of biological agent is preferably used. In one embodiment of the invention, the excess of biological agent is separated from the inoculated wood at the beginning of, at the end of, or during, the incubation period. This may be accomplished by removing the inoculated wood from the solution of biological agent, or by draining and collecting the biological agent from the inoculated wood. In either embodiment, the recovered excess biological agent may be discarded or recycled for subsequent use in the process of the invention.

In one embodiment of the invention, the wood is treated prior to the addition of the biological agent to remove, destroy or disable unwanted microorganisms and other non-biological agents and contaminates which be present in or on the wood. This pretreatment step may be performed before or during the compression step. Preferably, the pretreatment step comprises subjecting the wood substrate to steam; preferably low pressure steam, approximately 60 psig, applied for about 1 to 2 minutes at atmospheric pressure. Other agents may be used in the pretreatment step which have the function of removing, destroying or disabling such microorganisms and other non-biological agents and contaminants; provided, however, that care should be taken to ensure that any such pretreatment agents will not interfere with the subsequent biological agent treatment process or the later pulp refining and paper manufacturing processes. Such alternative agents may include hot water, dry heat, gamma, ultraviolet and infrared radiation, and gas-treatment, for example with ethylene oxide.

Addition of the biological agent to the compressed wood, or to wood which has been compressed and fully or partially decompressed, produces the inoculated wood. The inoculated wood is then incubated for sufficient time and under suitable conditions to allow for the desired structural and/or compositional alterations in the wood to occur. Preferably, the incubation period is about 60 seconds to about 14 days; more preferably 1 to 10 days; still more preferably 1 to 7 days. When the biological agent comprises enzymes, a period of about 1 to about 48 hours, preferably about 1 to about 48 hours, more preferably about 2 to about 6 hours is a suitable incubation period. The precise time, temperature and other parameters of the incubation period will vary depending on the nature of the biological agent used in the process of the invention. The incubation parameters will also vary depending on the nature and degree of structure and composition alteration desired; i.e., the final percentages of lignin, cellulose, hemicellulose and/or resins in the wood to be degraded or the consistency of the pulp desired. Determination of the desired parameters are within the ability of the skilled practitioner in the art.

The medium of biological agent comprises a solution or suspension comprising one or more strain of fungi or bacteria, culture products from such fungi or bacteria, such as enzymes, substances obtained from such cultures, enzymes from non-microbial sources, or combinations thereof. The fungi or bacteria suitable for use in the process of the present invention may be any now known or subsequently discovered in nature or bioengineered which have activity in the degradation or alteration of wood material substrates, for example wood chips and the like. As is particularly applicable to the paper manufacturing industry, this includes genera, species and strains of fungi and bacteria which have lipolytic, proteolytic, ligninolytic, cellulolytic and hemicellulolytic activity. Other genera, species or strains of fungi, bacteria, or other microorganisms with other composition or structure altering activity would be useful in the process of the invention as applied to other wood substrates for conversion to products useful in other applications. Suitable genera of fungi include, by way of example, *Ceriporiophsis*, *Phanerochaete* and *Ophiostoma*. Other examples of suitable microorganisms for use in the present invention are described in the following publications which are incorporated by reference herein: T. K. Kirk et al., "Enzymatic "Combustion": The Microbial Degradation of Lignin", Ann.Rev.Microbiol., **41**, 465-505 (1987); L. Jurasek et al., Biological Treatments of Pulps", Biomass, **15**, 103-108 (1988); K.-E. Erikson, "Breakthroughs in Biotechnology Show

Promise for Paper Industry", Pulp & Paper, 114 et seq. (May 1987); Seelenfreund et al., "Production of Soluble Lignin-rich Fragments (APPL) from Wheat Lignocellulose by *Streptomyces viridosporus* and Their Partial Metabolism by Natural Bacterial Isolates Precipitable Polymeric Lignin Degradation by *Pseudomonas* spp. and *Enterobacter* sp.", J. Biotechnol., **13** (2-3), 145-158 (1990); E.C. Setliff et al., "Screening White-Rot Fungi for Their Capacity to Delignify Wood", in Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications, T.K. Kirk et al, eds., (CRC Press, Inc., Boca Raton), Vol.1, Chap.7 (1980).

The biological agent may comprise culture products and/or substances obtained therefrom, comprising the various enzymes and other substances produced by the fungi or bacteria or resulting from the interaction and reaction of such enzymes and other substances with components in or added to the culture media. These culture products and substances obtained therefrom may comprise, for example, lipolytic, proteolytic, ligninolytic, cellulolytic and hemicellulolytic enzymes; e.g., lipase, protease, cellulase, hemicellulase, ligninase and laccase. Such enzymes may be present in the medium of biological agent as culture products of the fungal or bacterial culture employed. Alternatively or additionally, they may be present therein as a result of extraction from biological culture media or from non-microbial sources. In the latter circumstance, they may be added to a solution of fungi or bacteria to augment the naturally produced culture products or they may be used individually or in combination in solution as the "biological agent" in the "medium comprising biological agent".

In one embodiment of the invention, the medium of biological agent used in the process of the invention further comprises additional components to aid in the composition or structure altering process. These additional components may include, for example, nutrients, growth stimulators, buffers, surfactants and other promoters which may promote the growth, activity or penetration of the biological agents employed or may improve their distribution throughout the wood substrate. Examples of such components would include minerals or sugars as nutrients. In this embodiment, such components are delivered into the substrate together with the biological agent as desired.

The preparation of the medium comprising biological agent is within the ability of the skilled practitioner in the art. The nature of the medium, whether aqueous or otherwise, and the concentration or level or activity of biological agent in the medium will varying depending

on the biological agent employed and the extent of structural and/or compositional alteration of the substrate desired. Again, determination of those parameters is within the ability of the skilled practitioner in the art.

EXAMPLES

The invention may be illustrated by the following examples:

Example 1

Mill chipped southern Loblolly pine chips are sprayed with an aqueous suspension of $1x10^9$ fungal spores per 50g of o.d. chips. The fungus employed is a strain of *Ceriporiopsis subvermispora* which has known ligninase activity. These inoculated chips are allowed to stand at ambient temperature for 2 weeks.

After 2 weeks, the inoculated chips are made into mechanical pulp by refining first in a Model 418 Andritz Sprout-Bauer primary stage pressurized refiner to 600 ml freeness and then in a Model 401 secondary atmospheric pressure refiner to levels in the range of 100 to 200 ml freeness.

These pulps are made into TAPPI handsheets resulting in the strength properties here determined at 150 ml freeness. These are shown in Table A referred to as "Baseline".

TABLE A

COMPARISON OF 2 WEEK TREATMENT BY SPRAYING OF FUNGI TO THE PRESENT INVENTION

PROCESS	BASELINE	NEW PROCESS
Incubation Period, Days	14	8
Refining Energy, HPD/T		
Primary Stage	60	55
Secondary Stage	60	60
Total	120	115
Freeness, ml	150	150
Handsheet Properties		
Breaking Length, km	3.2	3.2
Burst Factor, m^2/cm^2	17	17
Tear Factor, dm^2	82	82

Example 2

200 o.d. pounds of untreated wood chips from the source in Example 1 are presteamed at atmospheric pressure for 2 minutes using 60 psig steam.

These sterilized chips are then processed through an Andritz Sprout-Bauer Model 560GS IMPRESSIFINER® employing a 4:1 compression ratio. After the impregnation zone, the destructured compressed chips are allowed to expand into an aqueous suspension of *Ceriporiopsis subvermispora* of sufficient concentration to result in the uptake of $1x10^9$ fungal spores per 50g of o.d. chips. The treated chips are drained of free inoculating solution and allowed to incubate for 8 days at ambient temperature.

After 8 days, the inoculated chips are made into mechanical pulp by refining first in a Model 418 Andritz Sprout-Bauer primary stage pressurized refiner to 600 ml freeness and then in a Model 401 secondary stage atmospheric pressure refiner to three levels in the range of 100 to 200 ml freeness.

These pulps are made into TAPPI handsheets and strength properties were determined at 150 ml freeness. These are shown in Table A referred to as "New Process".

This example demonstrates the ability of the new process to achieve equal pulp strength results in shorter incubation times. Reduction in the power needed to refine the wood to mechanical pulp is also demonstrated.

Example 3

400 o. d. pounds of mill chipped southern loblolly pine chips are presteamed at atmospheric pressure for 2 minutes and separated into four samples of 100 o. d. pounds each.

Sample 1 Chips are submerged in 30 liters of 10mM sodium acetate buffer, pH 4.5.

Sample 2 Chips are submerged in 30 liters of 10mM sodium acetate buffer, pH 4.5 to which 50,000 units of Clariant Cartazyme HS™ (5 grams at 10,000 units per gram) has been added.

Sample 3 Chips are processed through an Andritz Sprout-Bauer Model 560GS IMPRESSIFINER employing a 4:1 compression ratio. After the impregnation zone, the destructured compressed chips are allowed to expand into 30 liters of 10mM sodium

acetate buffer, pH 4.5. The chips are then submerged in 30 liters of 10mM sodium acetate buffer, pH 4.5.

Sample 4 Chips are processed through an Andritz Sprout-Bauer Model 560GS IMPRESSIFINER employing a 4:1 compression ratio. After the impregnation zone, the destructured compressed chips are allowed to expand into 30 liters of 10mM sodium acetate buffer, pH 4.5 to which 50,000 units of Clariant Cartazyme HS™ (5 grams at 10,000 units per gram) has been added. The chips are then submerged in 30 liters of 10mM sodium acetate buffer, pH 4.5 containing the same concentration of enzyme.

All four samples are incubated at 50°C for 48 hours. The liquid is drained from the chips and the chips are made into mechanical pulp by refining first in a Model 418 Andritz Sprout-Bauer primary stage pressurized refiner to 600 ml freeness and then in a Model 401 secondary stage atmospheric pressure refiner to three levels in the range of 100 to 200 ml freeness.

These pulps are made into TAPPI handsheets and strength properties are determined at 150 ml freeness. The results are shown in Table B. This example illustrates how the invention could be used to reduce the amount of power required to refine the wood to mechanical pulp while improving pulp strength.

TABLE B

COMPARISON OF ENZYME TREATED AND UNTREATED AS WELL AS COMPRESSED AND NONCOMPRESSED CHIPS

	Sample 1	Sample 2	Sample 3	Sample 4
Refining Energy, HPD/T				
Primary Stage	60	60	56	51
Secondary stage	60	60	60	60
Total	120	120	116	111
Freeness, ml	150	150	150	150

Handsheet Properties				
Breaking Length, km	2.7	2.9	2.7	3.2
Burst Factor, m^2/cm^2	14	15	14	17
Tear Factor, dm^2	70	74	70	82

Example 4

400 o. d. pounds of mill chipped southern loblolly pine chips are presteamed at atmospheric pressure for 2 minutes and separated into four samples of 100 o. d. pounds each.

Sample 1 Chips are submerged in 30 liters of 10mM sodium phosphate buffer, pH 7.5.

Sample 2 Chips are submerged in 30 liters of 10mM sodium phosphate buffer, pH 7.5 to which 50,000 units of Clariant Cartazyme NS™ (50 grams at 1000 units per gram) and 350,000 units of Sigma porcine pancreas Lipase L-3126 (5 grams at 70,000 units per gram) have been added.

Sample 3 Chips are processed through an Andritz Sprout-Bauer Model 560GS IMPRESSIFINER employing a 4:1 compression ratio. After the impregnation zone, the destructured compressed chips are allowed to expand into 30 liters of 10mM sodium phosphate buffer, pH 7.5. The chips are then submerged in 30 liters of 10mM sodium phosphate buffer, pH 7.5.

Sample 4 Chips are processed through an Andritz Sprout-Bauer Model 560GS IMPRESSIFINER employing a 4:1 compression ratio. After the impregnation zone, the destructured compressed chips are allowed to expand into a solution of 10mM sodium phosphate buffer, pH 7.5 to which 50,000 units of Clariant Cartazyme NS™ (50 grams at 1000 units per gram) and 350,000 units of Sigma porcine pancreas Lipase L-3126 (5 grams at 70,000 units per gram) have been added. The chips are then submerged in 30 liters of 10mM sodium phosphate buffer, pH 7.5 containing the same concentration of enzymes.

All four samples are incubated at 42°C for 48 hours. The liquid is drained from the chips and the chips are made into mechanical pulp by refining first in a Model 418 Andritz Sprout-Bauer primary stage pressurized refiner to 600 ml freeness and then in a Model 401 secondary stage atmospheric pressure refiner to three levels in the range of 100 to 200 ml freeness.

These pulps were made into TAPPI handsheets and strength properties were determined at 150 ml freeness. The results are shown in Table C. This example illustrates how the invention could be used to reduce the amount of power required to refine the wood to mechanical pulp while improving pulp strength.

TABLE C

COMPARISON OF ENZYME TREATED AND UNTREATED AS WELL AS COMPRESSED AND NONCOMPRESSED CHIPS

	Sample 1	Sample 2	Sample 3	Sample 4
Refining Energy, HPD/T				
Primary Stage	60	60	54	45
Secondary stage	60	60	60	60
Total	120	120	116	105
Freeness, ml	150	150	150	150
Handsheet Properties				
Breaking Length, km	2.7	2.9	2.7	3.2
Burst Factor, m^2/cm^2	14	15	14	17
Tear Factor, dm^2	70	74	70	82

What is claimed:

1. A method for altering the structure or composition of wood comprising adding to compressed wood a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent, to produce inoculated wood upon decompression.

2. A method according to Claim 1 wherein said wood comprises wood chips.

3. A method according to Claim 1 wherein said wood is subjected to pressure by means of a compression screw device.

4. A method according to Claim 1 wherein said wood is subjected to pressure by means of a roll press device.

5. A method according to Claim 1 wherein said wood is subjected to pressure in the range of about 200 to about 10,000 psig.

6. A method according to Claim 5 wherein said pressure is applied for at least about 0.1 seconds.

7. A method according to Claim 1 wherein said biological agent comprises one or more fungi, one or more bacteria, one or more culture products of said fungi or bacteria, one or more substances obtained from said culture, one or more enzymes of microbial or non-microbial source, or combinations thereof.

8. A method according to Claim 7 wherein said fungi comprises at least one lignin-degrading fungi.

9. A method according to Claim 7 wherein said fungi is selected from the group consisting of the genera *Ceriporiophsis*, *Phanerochaete* and *Ophiostoma*.

10. A method according to Claim 7 wherein said biological agent comprises culture products or substances obtained therefrom produced by culturing at least one fungus in growth medium.

11. A method according to Claim 10 wherein said culture products or substances obtained therefrom comprise enzymes.

12. A method according to Claim 11 wherein said enzymes comprise lipolytic enzymes, proteolytic enzymes, ligninolytic enzymes, cellulolytic enzymes or hemicellulolytic enzymes.

13. A method according to Claim 1 wherein said wood is incubated after contact with said medium for a period of time of about 60 seconds to about 14 days.

14. A method according to Claim 1 wherein said wood is incubated in contact with said medium for sufficient time to remove at least a portion of lignin in said wood.

15. A method according to Claim 1 wherein said wood is incubated in contact with said medium for sufficient time to remove at least a portion of cellulose in said wood.

16. A method according to Claim 1 wherein said wood is incubated in contact with said medium for sufficient time to remove at least a portion of hemicellulose in said wood.

17. A method according to Claim 1 wherein said wood is incubated in contact with said medium for sufficient time to remove at least a portion of extractives in said wood.

18. A method according to Claim 1 wherein said wood is incubated in contact with said medium for sufficient time to hydrolyze at least a portion of covalent bonds in lignin, cellulose or hemicellulose, or combinations thereof, in said wood.

19. A method according to Claim 1 wherein said medium is maintained at a temperature in the range from about 10°C to about 100°C.

20. A method according to Claim 1 wherein said medium is maintained at a pH in the range from about 2 to about 11.

21. A method according to Claim 1 wherein said wood is contacted with excess medium for a period of at least about 5 seconds.

22. A method according to Claim 21 wherein said excess medium is removed from said wood after contacting said wood with said medium.

23. A method according to Claim 21 wherein said excess medium is removed from said wood after contacting said wood with said medium, and said excess medium is available to inoculate additional wood.

24. A method according to Claim 1 wherein said medium further comprises compounds suitable as nutrients, co-factors, surfactants and/or buffers for said biological agent.

25. A method according to Claim 1 wherein said medium further comprises compounds suitable as growth stimulators for said biological agent.

26. A method according to Claim 1 wherein said medium further comprises compounds suitable as dispersion agents to promote distribution of said biological agent.

27. A method according to Claim 1 further comprising a pretreatment step for the removal of undesirable microorganisms and other contaminants prior to contacting said wood with said media.

28. A method according to Claim 27 wherein said pretreatment step comprises subjecting said wood to steam.

29. A method according to Claim 28 wherein said pretreatment step is performed prior to subjecting said wood to pressure.

30. A method according to Claim 29 wherein said pretreatment step is performed immediately prior to subjecting said wood to pressure.

31. A method according to Claim 1 comprising an additional step of incubating said inoculated wood.

32. A method according to Claim 31 wherein said wood is incubated at a temperature of about 10°C to about 100°C.

33. A method according to Claim 31 wherein said wood is incubated for a period of time of about 60 seconds to about 14 days.

34. A method according to Claim 33 wherein said wood is incubated after contact with said medium for a period of time of about 1 to about 10 days.

35. A method according to Claim 34 wherein said wood is incubated after contact with said medium for a period of time of about 1 to about 7 days.

36. A method according to Claim 1 wherein said compressed wood is fully or partially decompressed prior to adding said medium.

37. A method for altering the structure or composition of wood chips comprising adding to compressed wood chips a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent comprising one or more fungi or bacteria, one or more culture products or said fungi or bacteria, one or more substances obtained from said culture, one or more enzymes from non-microbial sources, or combinations thereof, to produce inoculated wood chips upon decompression, wherein said inoculated wood chips are incubated for a period of time of about 60 seconds to about 14 days, at a temperature of about 10°C to about 100°C.

38. A method for altering the structure or composition of wood chips comprising the steps of subjecting said wood to pressure to produce compressed wood; inoculating said compressed wood by contacting said compressed wood with a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent, to produce inoculated wood.

39. A method according to Claim 38 wherein said wood comprises wood chips.

40. A method according to Claim 38 wherein said biological agent comprises one or more fungi, one or more bacteria, one or more culture products of said fungi or bacteria, one or more substances obtained from said culture, enzymes from non-microbial sources, or combinations thereof.

41. A method for altering the structure or composition of wood comprising adding to said wood, wherein said wood has been structurally-altered by compression and subsequent full or partial decompression, a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent, to produce inoculated wood.

42. A method for altering the structure or composition of wood chips comprising the steps of subjecting said wood to pressure to produce compressed wood; relaxing said compressed wood to produce fully or partially decompressed wood; inoculating said decompressed wood by contacting said decompressed wood with a medium comprising a structure-altering or composition-altering effective amount of at least one biological agent, to produce inoculated wood.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06974

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12S 3/04
US CL :435/277
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/277, 278; 162/17-19, 56, 70, 71

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,374,555 A (POKORA ET AL.) 20 December 1994 (20.12.94), see entire document.	1-3, 5-7, 10-14, 18-21, 24, 26, 31-33, 36-42 --- 1-42
Y	US 3,962,033 A (ERIKSSON ET AL.) 08 June 1976 (08.06.76), see entire document.	1-42
Y	US 3,471,365 A (ASPLUND) 07 October 1969 (07.10.69), see entire document.	1-42
Y	BOHN. "Alkaline peroxide mechanical pulping: High yield pulp for the 90's." TAPPI PROCEEDINGS. 1990. pages 47-52, see entire document.	1-42

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06974

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JP 57-143591 A (HOKUETSU SEICHI KK) 04 September 1982 (04.09.82), see entire document.	4
Y	EP 0 430 915 A1 (ENSO-GUTZEIT OY) 05 June 1991 (05.06.91), see entire document.	15, 16
Y	US 3,486,969 A (NILSSON ET AL.) 30 December 1969 (30.12.69), see entire document.	17